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ORIGINAL ARTICLE

Clinical and Laboratory Analysis of Influenza B Infection in Children in Taichung, Taiwan during the 2006–2007 Flu Season

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Background: An epidemic of influenza B/Malaysia/2506/2004 was reported in Taiwan during the 2006–2007 flu season. We investigated the flu vaccination history and clinical and laboratory characteristics of children with influenza B infection.
Methods: We enrolled children younger than 18 years old who visited one of two hospitals between November 2006 and February 2007 with influenza-like illnesses. Throat swabs were taken on their first visit and cultured for viruses. Complete and differential blood counts and blood biochemical parameters were analyzed in some children.
Results: Influenza virus was cultured from 51.0% of patients (101/198), 87 (86.1%) of who were infected with influenza virus type B. The remaining 14 (13.9%) were infected with influenza virus type AH3. The 87 children (median age 7.8 years) with culture-proven influenza B virus infection were enrolled. Nine parents reported that enrolled children had been vaccinated against influenza. Leukopenia was found in 56.1% (32/57) of patients, leukocytosis in 3.5% (2/57), and thrombocytopenia in 1.8% (1/57). Thirteen of 23 patients (56%) tested for creatine kinase (CK) had elevated levels (>160 U/L), and 11 of 23 (47.8%) had myalgia associated with raised CK ($p<0.05$). Twenty-six children developed complications, including one case of pneumonia with acute respiratory distress syndrome and one case of flu-associated encephalitis.
Conclusion: Most children who contracted influenza B infection had not been vaccinated. Almost half the children had leukopenia, and some had leukocytosis, but thrombocytopenia was rare. There was a significant association between raised CK levels and myalgia in influenza B infection.

1. Introduction

The influenza virus responsible for the 1918 epidemic was extremely virulent and caused many deaths due to secondary bacterial pneumonia. Viral pneumonia is able to kill previously healthy young individuals within 2 days. Every year, 20% of children worldwide develop influenza. Children shed virus at higher

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titers and for longer periods than adults.[1] Influenza is generally a self-limited respiratory tract illness in children, and severe complications have been reported in only a few cases. However, influenza-associated encephalitis and deaths in previously healthy children have been increasingly reported.[2,3]

An island-wide epidemic of influenza B/Malaysia/2506/2004 was reported in Taiwan during the 2006–2007 flu season. Many children suffered from high spiking fever, general malaise and muscle aches, and required medical help. Sixteen cases of influenza B associated with complications were reported, including five deaths. Four of the five children who died were under 18 years old.[4] In this study, we retrospectively and prospectively enrolled children with influenza B infection to investigate the flu vaccination history and the clinical and laboratory characteristics of flu in children and adolescents.

2. Materials and Methods

Children younger than 18 years of age with influenza-like illness (ILI) who visited the emergency room or outpatient clinic, or who were hospitalized at Taichung Veterans General Hospital (TCVGH) or Da-Li Jen-Ai Hospital (DLJAH) from November 2006 to February 2007 were enrolled in this study. ILI was defined on the basis of sudden onset of fever ($>37.8^{\circ}C$) and any signs or symptoms of acute respiratory infection such as cough, sore throat, or a runny or stuffy nose.[5] A throat swab was taken on their first visit and transferred to the laboratory for virus culture and immune staining (Chemicon). Throat swab specimens were collected and placed in transportation medium (Copan), and the medium was filtered with a 0.45-μm filter after thorough squeezing out of the swab. Two hundred microliters of each specimen were inoculated into tubes containing Madin-Darby canine kidney (MDCK) cells and Hep-2 cells for influenza and other respiratory virus isolation. The tubes were incubated for up to 14 days to observe any cytopathic effects (CPE). All influenza isolates were typed using the indirect immunofluorescence antibody method (Chemicon). A positive CPE was confirmed by typing virally-infected cells using monoclonal antibodies for influenza A and B. Influenza virus type A isolates were further classified as AH3 or AH1 by multiplex reverse transcription-polymerase chain reaction. Patients with virus culture-confirmed influenza B infection were enrolled for further analysis and study. Blood samples were also obtained from some patients.

Laboratory data, including complete and differential blood counts (CBC/DC), alanine transaminase (ALT), creatine kinase (CK), creatinine and C-reactive protein (CRP) were analyzed. CBC/DC was performed automatically using a Coulter LH750 analyzer (Beckman Coulter, Inc., Fullerton, CA, USA). Biochemical tests were performed using Hitachi 7180 and 7600 autoanalyzers (Hitachi High-Technologies Corporation, Japan). CRP was analyzed by immunonephelometry. Leukopenia was defined as a white blood count (WBC) $<6000/\mu L$ for children aged 6 months to 6 years, $<4500/\mu L$ for children aged 7–12 years, or $<5000/\mu L$ for adolescents aged 13–18 years.[6] Leukocytosis was defined as a WBC $>15{,}000/\mu L$ for children aged 6 months to 6 years, $>13{,}500/\mu L$ for children aged 7–12 years, or $>10{,}000/\mu L$ for adolescents aged 13–18 years.[6] Thrombocytopenia was defined as a platelet count $<100{,}000/\mu L$. Thrombocytosis was defined as a platelet count $>400{,}000/\mu L$. Anemia was defined as hemoglobin (Hb) $<12.3\,g/dL$ in males or $<11.3\,g/dL$ in females.

After giving informed consent, parents were prospectively or retrospectively requested to complete a questionnaire on their first visit, or by chart review and telephone follow-up. The questionnaire included questions regarding flu vaccination history (how many doses of flu vaccine the patient received, flu-like illnesses since they received flu vaccine), underlying diseases, and clinical symptoms of flu (duration of fever, headache and myalgia).

Children with any infiltrates on chest radiographs during the influenza episode were identified, and the chest radiographs of these children were reevaluated by a radiologist for verification of pneumonia infiltrates. The diagnosis of influenza pneumonia was based on simultaneous finding of laboratory-documented influenza and infiltrates compatible with pneumonia on the chest radiograph.[7] Influenza-associated myositis (IAM)[8] was defined as virologically-proven influenza or influenza-like illness plus clinical evidence of localized myalgia, and laboratory evidence of elevated serum CK.

2.1. Statistical analysis

We compared factors associated with flu vaccination using Fisher's exact test. The relationships between myalgia and CK, and influenza B infection were analyzed using the χ^2 test. A p value <0.05 was defined as statistically significant.

3. Results

3.1. Positive culture rates in patients with influenza-like illnesses

A total of 198 throat swab specimens were collected for virus culture from 196 subjects with an ILI during the 2006–2007 flu season. The rate of

Table 1 Culture-positive rate of 198 specimens from patients with influenza-like illnesses

Virus	Isolation positive rate (%)
Influenza B virus	87/198 (43.9)
Adenovirus	18/198 (9)
Influenza A (H3) virus	14/198 (7)
HSV type 1	3/198 (1.5)
Nonpolio enterovirus	2/198 (1.0)
Parainfluenza virus type 3	1/198 (0.5)

HSV = herpes simplex virus.

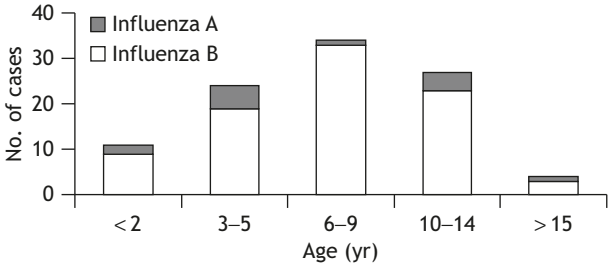

Figure Age distribution of patients with influenza A and influenza B infection.

laboratory-confirmed influenza infection was 51.0% (101/198) according to cell culture findings (Table 1). Among patients with positive cultures, 87 (86.1%) were infected with influenza virus type B, while the remaining 14 (13.9%) were infected with influenza virus type AH3. From the total 198 swabs, 9% (18/198) were positive for adenovirus infection, 1.5% (3/198) for herpes simplex virus (HSV) type 1 infection, 1.0% (2/198) for nonpolio enterovirus infection, and 0.5% (1/198) for parainfluenza virus type 3 infection (Table 1).

3.2. Patient characteristics

A total of 100 children younger than 18 years of age with culture-proven influenza A and B virus infection were enrolled. Their age distribution is shown in Figure. Among these, 87 children had culture-proven influenza B virus infection. Seventy of these were treated at TCVGH, and the other 17 subjects were treated at DLJAH (age range: 9 months to 16 years). Most children were between 6 and 9 years old. The median age was 7.8 years.

3.3. Vaccination against influenza

Flu vaccination histories were completed by 87.4% (76/87) of families (parent or caregiver). The 11 nonresponses were due to wrong phone numbers or unsuccessful contact. In TCVGH, 81.4% (57/70) of patients had not received a seasonal influenza vaccination, compared with 58.8% (10/17) in DLJAH. Ten percent (7/70) of patients in TCVGH and 11.8% (2/17) in DLJAH had received a seasonal influenza vaccination. We analyzed the relationships between age and fever duration, and vaccination against influenza using Fisher's exact test. Comparing children aged ≤ 6 years and those aged >6 years, $z=1.10874$. Comparing fever durations of <5 days and ≥ 5 days, $z=-0.5698$. There was no significant difference in the influenza vaccination rates according to age and fever duration ($z>-1.96$).

3.4. Laboratory analysis

Blood tests were performed in 57 of the 87 patients with influenza B infection (49 in TCVGH, 8 in DLJAH). The results of CBC/DC, CRP, CK, ALT and creatinine were analyzed (Table 2). Leukopenia was found in 56.1% (32/57) of patients, leukocytosis 3.5% (2/57), thrombocytopenia in 1.8% (1/57), thrombocytosis in 3.5% (2/57), and anemia in 8.8% (5/57).

Twenty-three patients were tested for CK, and 13 of them (56.5%) in TCVGH had raised levels (>160 U/L) (Table 2). Eleven of the patients (84.6%) with elevated CK levels, compared with only four (40%) of the patients with normal CK levels (Table 3) had myalgia. One of the 11 patients with IAM was a 6-year-old boy who also had rhabdomyolysis. In this patient on hospital day 1, CK was elevated to 17,697 U/L, ALT was 75 U/L, blood urea nitrogen was 13 mg/dL and creatinine was 0.7 mg/dL. On hospital day 2, CK was elevated to 21,084 U/L, AST was 601 U/L and ALT was 154 U/L. On hospital day 4, CK was 1132 U/L, AST was 79 U/L and ALT was 104 U/L. Variables associated with influenza B infection were analyzed by χ^2 test. There was a significant association between CK >160 U/L and myalgia in influenza B infection ($p>0.05$).

3.5. Hospitalization and complications

A total of 31 children with influenza B infection were hospitalized, including 22 in TCVGH and 9 in DLJAH. Twenty-six children had complication in TCVGH, including seven with myositis, one with acute otitis media (AOM), one with acute bronchiolitis, one with croup, 16 with pneumonia, one with pneumonia with acute respiratory distress syndrome (ARDS) and one with viral encephalitis. Pneumonia was diagnosed in 17/87 (19.5%) of our patients, and the hospitalization rate was 41.2% (7/17).

4. Discussion

The rate of laboratory-confirmed influenza B infection in our study was 43.7% (87/198), according

Table 2 Analysis of laboratory findings in patients with influenza B infection

Test	Case number	Definition	Positive (%)
WBC	57	Leukopenia (WBC < 6000/μL for children aged 6 months to 6 years or WBC < 4500/μL for children aged 7–12 years or WBC < 5000/μL for adolescents aged 13–18 years)	32 (56.1)
	57	Leukocytosis (WBC > 15,000/μL for children aged 6 months to 6 years or WBC > 13,500/μL for children aged 7–12 years or WBC > 10,000/μL for adolescent aged 13–18 years)	2 (3.5)
Platelet	57	Thrombocytosis (platelets > 400,000/μL)	2 (3.5)
	57	Thrombocytopenia (platelets < 100,000/μL)	1 (1.8)
Hb	57	Anemia (Hb < 12.3 g/dL in male or < 11.3 g/dL in female)	5 (8.8)
CK	23	Increased (CK > 160 U/L)	13 (56.5)
ALT	43	Increased (ALT > 44 U/L)	9 (20.9)
Creatinine	43	Increased (Creatinine > 1.4 mg/dL)	0 (0)
CRP	57	Increased (CRP > 0.5 mg/dL)	25 (43.9)

ALT = alanine transaminase; CRP = C-reactive protein; CK = creatine kinase; Hb = hemoglobin; WBC = white blood count.

Table 3 Relationship between myalgia and CK levels in children with influenza B infection

Myalgia	Case number	CK > 160 U/L	CK < 160 U/L	Note
Yes	15	11 (47.8%)	4 (17.4%)	
No	8	2 (8.7%)	6 (26.1%)	
Total	23	13	10	$p < 0.05$

CK = creatine kinase.

to viral culture findings. Of note, our samples were collected from subjects with flu-like illnesses, which would be expected to increase the probability of influenza virus detection. A previous study that included children younger than 36 months presenting with fever onset (≥ 38°C) during the peak of the epidemic detected influenza virus in nearly half of the febrile children (283/575, 49%).[9] Among these, 253 (89%) were infected with influenza virus type A/H3N2, while the remaining 30 (11%) were infected with influenza virus type B.

In our study, nine children were infected with influenza B despite having received a flu vaccination. Since 2004, the Advisory Committee on Immunization Practices has recommended that all children aged 6–23 months should receive two doses of trivalent inactivated influenza vaccine.[10] Three children aged 6–23 months received one dose of influenza vaccine in our study. The duration of fever was less than 4 days, except in one patient, who had fever for 8 days. There were no sequelae in these patients. Generally, individuals who are vaccinated against influenza should be protected, even though the strain coverage of the vaccine may not be fully compatible.

The peripheral WBC in influenza B infection can be variable, ranging from normal counts to leukocytosis or leukopenia.[11] Leukopenia was found in 56.1% (32/57) of our patients with influenza B infection, 12 of who had complications. Nine patients developed pneumonia, one developed acute bronchiolitis, one developed rhabdomyolysis, and five developed myositis, but there were no sequelae in these patients. Joan et al studied the mechanisms behind influenza A virus-induced leukopenia, with emphasis on the potential induction of apoptosis of lymphocytes by the virus.[12] Leukocytosis was found in 3.5% (2/57) of our patients, one of who developed pneumonia with ARDS. He was a 14-year-old male patient with underlying chronic lung disease post tracheostomy. CBC/DC showed a WBC of 27,200/μL, Hb of 8.7 g/dL, a platelet count of 327,000/μL, and CRP of 2.6 mg/dL on hospital day 1. His pneumonia was treated with aqueous penicillin. He also had IAM. A finding of leukocytosis should alert clinicians to the potential complications of influenza B, such

as secondary bacterial infection or AOM. Secondary bacterial pneumonia is most commonly caused by *Streptococcus pneumoniae* or *Staphylococcus aureus*.[7,13] Only one patient in our study had thrombocytopenia, which is an uncommon finding in influenza B infections in children.[11]

In the current study, IAM occurred in 11 of 23 (47.8%) patients, including one patient with rhabdomyolysis. The rate of benign acute childhood myositis in influenza B was reported to be 33.9%.[14] IAM typically occurs in school-aged children, with a 2:1 male predominance. Blood CK concentration is almost invariably elevated and indicates muscle damage. Agyeman et al analyzed 316 cases of IAM.[8] Rhabdomyolysis occurred in 10 of 316 patients (3%), was more common in girls (80%), was more often associated with influenza A (86%), and led to renal failure in eight patients (80%).[8] It is thought that increased renal vascular resistance secondary to increased sympathetic tone, activation of the renin-angiotensin axis, increased concentrations of vasopressin, or glomerular microthrombi results in decreased renal blood flow. Finally, obstruction of the renal tubules by myoglobin, protein, or uric acid crystals may play a role in the development of acute renal failure.[15]

Although epidemiologic, clinical and laboratory data have established a firm link between influenza virus infection and IAM, the mechanisms by which the virus leads to muscle involvement are poorly understood. The two most commonly proposed mechanisms in the literature are direct muscle invasion by viral particles, and immune-mediated muscle damage triggered by the virus. The hypothesis of direct muscle invasion was supported by the findings of Agyeman et al[8] who isolated influenza virus from a muscle biopsy specimen in a child with IAM.

Pneumonia was diagnosed in 17/87 (19.5%) of our patients. Peltola et al reported that pneumonia occurred in 9% of their patients with influenza A or B infection,[16] and the rate of influenza-related pneumonia was estimated to be 14% in a retrospective survey of both outpatient and hospitalized children with influenza.[7] Influenza pneumonia may be underestimated, as the illness is difficult to differentiate from uncomplicated influenza and other respiratory infections, solely on the basis of clinical symptoms.[7]

Viral encephalitis occurred in a 2.8-year-old boy in our study. He was otherwise healthy, though his cousin had recently died of influenza B infection complicated with myocarditis. The clinical outcome was favorable. A national survey in Japan investigated the various parameters of the disease outbreak that occurred during the winter of 1998–1999. A total of 148 cases were diagnosed as influenza-associated encephalitis.[3] Of these, 130 (87.8%) were type A influenza and 17 were type B. Encephalitis mainly developed in children under 5 years. Multiple organ failure developed in many patients, and the mortality (31.8%) and morbidity were high. Influenza B infections can cause significant mortality and morbidity in immunocompromised children who have underlying medical conditions.

In our study, most children who contracted influenza B infections had not received an influenza vaccination during the flu season. Leukopenia was found in almost half of the children, while leukocytosis was found in some, but thrombocytopenia was uncommon. It is important to recognize the significance of the hematologic findings associated with influenza B infection. There was a significant association between raised CK levels and myalgia in children with influenza B infection.

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